Selective Modulators of Peroxisome Proliferator Activated Receptor-gamma, and Methods for the Use Thereof

FIELD OF THE INVENTION

The present invention relates to methods for the modulation of nuclear receptor mediated processes. In a particular aspect, the present invention relates to the use of a specific class of compounds for the modulation of processes mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ). In another aspect, the present invention relates to methods of testing compounds for their ability to regulate transcription-activating effects of PPAR-γ.

BACKGROUND OF THE INVENTION

Peroxisome proliferators are a structurally diverse group of compounds which, when administered to rodents, elicit dramatic increases in the size and number of hepatic and renal peroxisomes, as well as concomitant increases in the capacity of peroxisomes to metabolize fatty acids via increased expression of the enzymes required for the ß-oxidation cycle (Lazarow and Fujiki, *Ann. Rev. Cell Biol.* 1:489-530 (1985); Vamecq and Draye, *Essays Biochem.* 24:1115-225 (1989); and Nelali et al., *Cancer Res.* 48:5316-5324 (1988)). Chemicals included in this group are the fibrate class of hypolipidermic drugs, herbicides, and phthalate plasticizers (Reddy and Lalwani, *Crit. Rev. Toxicol.* 12:1-58 (1983)). Peroxisome proliferation can also be elicited by dietary or physiological factors such as a high-fat diet and cold acclimatization.

Insight into the mechanism whereby peroxisome proliferators exert their pleiotropic effects was provided by the identification of a member of the nuclear hormone receptor superfamily activated by these chemicals (Isseman and Green, *Nature* 347-645-650 (1990)). This receptor, termed peroxisome proliferator activated receptor alpha (PPARα), was subsequently shown to be activated by a variety of medium and long-chain fatty acids and to stimulate expression of the genes encoding rat acyl-CoA oxidase and hydratase-dehydrogenase (enzymes required for peroxisomal ß-oxidation), as well as rabbit cytochrome P450 4A6, a fatty acid ω-hydroxylase (Gottlicher et al., *Proc. Natl. Acad. Sci. USA* 89:4653-4657 (1992); Tugwood et al., *EMBO J.* 11:433-439 (1992); Bardot et al., *Biochem. Biophys. Res. Comm.* 192:37-45 (1993); Muerhoff et al., *J. Biol. Chem.* 267:19051-19053 (1992); and Marcus et al., *Proc. Natl. Acad. Sci. USA* 90(12):5723-5727 (1993).

The above-noted references suggest a physiological role for PPARα in the regulation of lipid metabolism. PPARα activates transcription by binding to DNA sequence elements, termed peroxisome proliferator response elements (PPRE), as a heterodimer with the retinoid X receptor. The retinoid X receptor is activated by 9-*cis* retinoic acid (see Kliewer et al., *Nature* 358:771-774 (1992), Gearing et al., *Proc. Natl. Acad. Sci. USA* 90:1440-1444 (1993), Keller et al., *Proc. Natl. Acad. Sci. USA* 90:2160-2164 (1993), Heyman et al., *Cell* 68:397-406 (1992), and Levin et al., *Nature* 355:359-361 (1992)). Since the PPARα-RXR complex can be activated by peroxisome proliferators and/or 9-*cis* retinoic acid, the retinoid and fatty acid signaling pathways are seen to converge in modulating lipid metabolism.

Since the discovery of PPARα, additional isoforms of PPAR have been identified, e.g., PPARβ, PPARγ and PPARδ, which are spatially differentially expressed. Because there are several isoforms of PPAR, it would be desirable to identify compounds which are capable of selectively interacting with only one of the PPAR isoforms. Such compounds would find a wide variety of uses, such as, for example, in the prevention of obesity, for the treatment of diabetes, and the like.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have identified a class of compounds which are capable of selectively modulating processes mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ). The identification of such compounds makes possible the selective intervention in PPAR-γ mediated pathways, without exerting inadvertent effects on pathways mediated by other PPAR isoforms.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 illustrates the activation of a GAL4-PPARγ fusion protein by a variety of prostaglandin or prostaglandin-like compounds. In the figure, black bars represent 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 (15-d PGJ2), the dark, striped bars represent prostaglandin-J_2 (PGJ2), the darkly shaded bars represent 9α,11β-prostaglandin-F_2 (9a,11bPGF2), the light, closely (diagonally) striped bars represent prostaglandin-I_2 (PGI2), the open bars represent prostaglandin-A_2 (PGA2), the dark bars with light dots represent prostaglandin-B_2 (PGB2), the horizontally hatched bars represent prostaglandin-D_2 (PGD2), the light bars with dark dots represent prostaglandin-E_2 (PGE2), the light, sparsely (diagonally) hatched bars represent prostaglandin-$F_{2\alpha}$ (PGF2a), and the

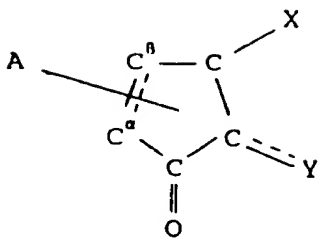
light bars with sparsely spaced dots represent bicycloprostaglandin-E_1 (BicycloE1).

Figure 2 illustrates the dose response for activation of a GAL4-PPARγ fusion protein by a variety of prostaglandin or prostaglandin-like compounds. In the figure, open circles represent prostaglandin-D_2 (PGD2), darkened circles represent prostaglandin-J_2 (PGJ2), open squares represent Δ^{12}-prostaglandin-J_2 (Δ12-PGJ2), and darkened squares represent 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 (15-deoxy-Δ12,14-PGJ2).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there are provided methods for modulating process(es) mediated by peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising conducting said process(es) in the presence of at least one PPAR-γ-selective prostaglandin or prostaglandin-like compound or precursor thereof.

PPAR-γ-selective prostaglandins or prostaglandin-like compounds contemplated for use in the practice of the present invention include members of the prostaglandin-J_2 family of compounds (e.g., prostaglandin-J_2, Δ^{12}-prostaglandin-J_2 or 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2), members of the prostaglandin-D_2 family of compounds (e.g., prostaglandin-D_2), or precursors thereof, as well as compounds having the structure I:



(I)

wherein:

A is selected from hydrogen or a leaving group at the α- or β- position of the ring, or A is absent when there is a double bond between C^{α} and C^{β} of the ring;

X is an alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl group having in the range of 2 up to 15 carbon atoms; and

Y is an alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl or substituted alkynyl group having in the range of 2 up to 15 carbon atoms.

As employed herein, the term "leaving group" refers to functional groups which can readily be removed from the precursor compound, for example, by nucleophilic displacement, under E_2 elimination conditions, and the like. Examples include hydroxy groups, alkoxy groups, tosylates, brosylates, halogens, and the like.

As employed herein, "lower alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 4 carbon atoms; "alkyl" refers to straight or branched chain alkyl groups having in the range of about 1 up to 12 carbon atoms; "substituted alkyl" refers to alkyl groups further bearing one or more substituents such as hydroxy, alkoxy (of a lower alkyl group), mercapto (of a lower alkyl group), halogen,

trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl, sulfonamide, and the like.

As employed herein, "cycloalkyl" refers to cyclic ring-containing groups containing in the range of about 3 up to 8 carbon atoms, and "substituted cycloalkyl" refers to cycloalkyl groups further bearing one or more substituents as set forth above.

As employed herein, "alkenyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon double bond, and having in the range of about 2 up to 12 carbon atoms and "substituted alkenyl" refers to alkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "alkynyl" refers to straight or branched chain hydrocarbyl groups having at least one carbon-carbon triple bond, and having in the range of about 2 up to 12 carbon atoms, and "substituted alkynyl" refers to alkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "aryl" refers to aromatic groups having in the range of 6 up to 14 carbon atoms and "substituted aryl" refers to aryl groups further bearing one or more substituents as set forth above.

As employed herein, "alkylaryl" refers to alkyl-substituted aryl groups and "substituted alkylaryl" refers to alkylaryl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkyl" refers to aryl-substituted alkyl groups and "substituted arylalkyl" refers to arylalkyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkenyl" refers to aryl-substituted alkenyl groups and "substituted arylalkenyl" refers to arylalkenyl groups further bearing one or more substituents as set forth above.

As employed herein, "arylalkynyl" refers to aryl-substituted alkynyl groups and "substituted arylalkynyl" refers to arylalkynyl groups further bearing one or more substituents as set forth above.

As employed herein, "aroyl" refers to aryl-carbonyl species such as benzoyl and "substituted aroyl" refers to aroyl groups further bearing one or more substituents as set forth above.

As employed herein, "heterocyclic" refers to cyclic (i.e., ring-containing) groups containing one or more heteroatoms (e.g., N, O, S, or the like) as part of the ring structure, and having in the range of 3 up to 14 carbon atoms and "substituted heterocyclic" refers to heterocyclic groups further bearing one or more substituents as set forth above.

As employed herein, "acyl" refers to alkyl-carbonyl species.

As employed herein, "halogen" or "halo" refers to fluoro substituents, chloro substituents, bromo substituents or iodo substituents.

In a presently preferred aspect of the present invention, "X" of Formula I is selected from:

$-(CRR)_m-Z$,

$-(CRR)_{m'}-C(R)=C(R)-(CRR)_{m'}-Z$, or

$-(CRR)_{m''}-C\equiv C-(CRR)_{m''}-Z$, wherein:

each R is independently selected from H, lower alkyl, substituted lower alkyl,

hydroxy, lower alkoxy, thioalkyl, halogen, trifluoromethyl, cyano, nitro, amino, carboxyl, carbamate, sulfonyl or sulfonamide,

m falls in the range of 1 up to 15,

each m' falls independently in the range of 0 up to 12, with the proviso that the total chain length of the alkenyl moiety does not exceed 15 carbon atoms,

each m" falls independently in the range of 0 up to 12, with the proviso that the total chain length of the alkynyl moiety does not exceed 15 carbon atoms, and

Z is a polar, heteroatom-containing substituent.

Those of skill in the art can readily identify numerous groups which satisfy the requirement that Z be a polar, heteroatom-containing (i.e., O, N, S, or the like) substituent. Thus, Z can be selected from cyano, nitro, amino, carbamate, or a substituent having the structure:

$-CH_2OR'$, wherein R' is selected from H, alkyl, alkenyl, alkynyl, acyl, aryl, or the like;

$-C(O)R''$, wherein R" is selected from H, alkyl, substituted alkyl, alkoxy, alkylamino, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aryloxy, arylamino, alkylaryl, substituted alkylaryl, arylalkyl, substituted arylalkyl, heterocyclic, substituted heterocyclic or trifluoromethyl,

$-CO_2R'''$, wherein R''' is selected from H, alkyl, alkenyl, alkynyl, or the like;

-SR', -S(O)R', -$S(O)_2$R' or -$S(O)_2$NHR', wherein each R' is as defined above,

and the like.

Especially preferred compounds employed in the practice of the present invention are those wherein "X" of Formula I is

-CRR-C(R)=C(R)-$(CRR)_m$-Z, wherein:

each R is independently selected from H, lower alkyl, substituted lower alkyl, hydroxy, alkoxy (of a lower alkyl group), halogen, trifluoromethyl, amino, carboxyl or sulfonyl,

m falls in the range of 1 up to 6, and

Z is selected from -CH_2OH, -CH_2OAc, -CO_2H, -CO_2Me or -CO_2Et.

In another preferred aspect of the present invention, "Y" of Formula I is selected from:

=C(R)-$[C(R)=C(R)]_n$-$(CRR)_{n'}$-Z' **(II)**,

=C(R)-$[C\equiv C]_{n''}$-$(CRR)_{n'}$-Z' **(IIA)**,

=C(R)-CRR-CR(R')-$(CRR)_{n'}$-Z' **(III)**,

-$[C(R)=C(R)]_n$-$(CRR)_{n'}$-Z' **(IV)**, or

-$[C\equiv C]_n$-$(CRR)_{n'}$-Z' **(IVA)**,

wherein

each R is independently as defined above,

each R' is independently selected from H, lower alkyl, substituted lower alkyl or a leaving group,

Z' is selected from H, lower alkyl or substituted lower alkyl,

n falls in the range of 0 up to 4,

n' falls in the range of 2 up to 12, and

n" falls in the range of 1 up to 3.

Especially preferred compounds contemplated for use in the practice of the present invention include those wherein "Y" of Formula I is selected from:

$=C(R)-C(R)=C(R)-(CRR)_{n'}-Z'$ (II),

$=C(R)-CRR-CR(R')-(CRR)_{n'}-Z'$ (III), or

$-C(R)=C(R)-CR(R')-(CRR)_{n'}-Z'$ (IV), wherein

each R is independently as defined above,

each R' is independently as defined above,

Z' is selected from H, lower alkyl or substituted lower alkyl, and

n' falls in the range of 1 up to 6.

Presently most preferred compounds for use in the practice of the present invention include those wherein "Y" of Formula I is

$=C(R)-C(R)=C(R)-(CRR)_{n'}-Z'$ (II),

wherein each R is selected from H, lower alkyl or substituted lower alkyl, n is 1, n' falls in the range of about 2 up to 6, and Z' is selected from H or lower alkyl; or those wherein "Y" of Formula I is

$=C(R)-CRR-CR(R')-(CRR)_{n'}-Z'$ (III) or

$-C(R)=C(R)-CR(R')-(CRR)_{n'}-Z'$ (IV),

wherein each R is selected from H, lower alkyl or substituted lower alkyl, R' is selected from H, lower alkyl, or an hydroxy group, n is 1, n' falls in the range of about 2 up to 6, and Z' is selected from H or lower alkyl.

Referring to the structural formulae set forth above, prostaglandin-D_2 (Pg-D2) is described by Formula I (as set forth above), wherein A is 9-OH, Y is IV, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m is 3, Z' is methyl, n is 1 and n' is 4; prostaglandin-J_2 (Pg-J2) is described by Formula I, wherein A is absent, Y is IV, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m is 3, Z'

is methyl, n is 1 and n' is 4; Δ^{12}-prostaglandin-J_2 (Δ^{12}-Pg-J2) is described by Formula **I**, wherein A is absent, Y is **III**, each R is hydrogen, R' is hydroxy, Z is $-CO_2H$, m is 3, Z' is methyl, n is 1 and n' is 4; 15-deoxy-$\Delta^{12,14}$-prostaglandin-J_2 (15-deoxy-$\Delta^{12,14}$-Pg-J2) is described by Formula **I**, wherein A is absent, Y is **II**, each R is hydrogen, Z is $-CO_2H$, m is 3, Z' is methyl, n is 1 and n' is 4.

The above-described compounds can be readily prepared using a variety of synthetic methods, as are well known by those of skill in the art. For example, many of the above-described compounds can be prepared chemically or enzymatically, from the naturally occurring precursor, arachidonic acid.

As employed herein, the term "modulate" refers to the ability of a modulator for a member of the steroid/thyroid superfamily to either directly (by binding to the receptor as a ligand) or indirectly (as a precursor for a ligand or an inducer which promotes production of ligand from a precursor) induce expression of gene(s) maintained under hormone expression control, or to repress expression of gene(s) maintained under such control.

As employed herein, the phrase "processes mediated by PPARγ" refers to biological, physiological, endocrinological, and other bodily processes which are mediated by receptor or receptor combinations which are responsive to the PPAR-γ-selective prostaglandin or prostaglandin-like compounds described herein. Such processes include cell differentiation to produce lipid-accumulating cells, modulation of blood glucose levels and insulin sensitivity, regulation of leptin levels and subsequent feeding levels (for the control of satiety and/or appetite), regulation of thermogenesis and fatty

acid metabolism, regulation of fat levels for the treatment of lipodystrophies, control of cell differentiation for the treatment of myxoid liposarcomas, regulation of triglyceride levels and lipoproteins for the treatment of hyperlipidemia, modulation of genes expressed in adipose cells (e.g., leptin, lipoprotein, lipase, uncoupling protein, and the like), and the like.

In accordance with the present invention, modulation of processes mediated by PPARγ can be accomplished *in vitro* or *in vivo*. *In vivo* modulation can be carried out in a wide range of subjects, such as, for example, humans, rodents, sheep, pigs, cows, and the like.

PPAR-γ-selective prostaglandin or prostaglandin-like compounds contemplated for use in the practice of the present invention can be employed for both *in vitro* and *in vivo* applications. For *in vivo* applications, the invention compounds can be incorporated into a pharmaceutically acceptable formulation for administration. Those of skill in the art can readily determine suitable dosage levels when compounds contemplated for use in the practice of the present invention are so used.

In accordance with another embodiment of the present invention, there is provided a method of testing compound(s) for the ability to regulate the transcription-activating effects of a peroxisome proliferator activated receptor-gamma (PPAR-γ), said method comprising assaying for changes in the level of reporter protein present as a result of contacting cells containing said receptor and reporter vector with said compound;

wherein said reporter vector comprises:

(a) a promoter that is operable in said cell,

(b) a hormone response element, and

(c) a DNA segment encoding a reporter protein,

wherein said reporter protein-encoding DNA segment is operatively linked to said promoter for transcription of said DNA segment, and

wherein said hormone response element is operatively linked to said promoter for activation thereof.

Hormone response elements contemplated for use in the practice of the present invention are composed of at least one direct repeat of two or more half sites separated by a spacer of one nucleotide. The spacer nucleotide can be selected from any one of A, C, G or T. Each half site of response elements contemplated for use in the practice of the invention comprises the sequence

-RGBNNM-,

wherein

R is selected from A or G;

B is selected from G, C, or T;

each N is independently selected from A, T, C, or G; and

M is selected from A or C;

with the proviso that at least 4 nucleotides of said -RGBNNM- sequence are identical with the nucleotides at corresponding positions of the sequence -AGGTCA-. Response elements employed in the practice of the present invention can optionally be preceded by N_x, wherein x falls in the range of 0 up to 5.

Sub F1 Presently preferred response elements contain at least one copy (with one, two or three copies most common) of the minimal sequence:

AGGACA A AGGTCA (SEQ ID NO:4).

As noted above, the minimal sequence can optionally be flanked by additional residues, for example, as in the sequence:

GGACC AGGACA A AGGTCA CGTTC (SEQ ID NO:5).

In a preferred embodiment of the present invention, only the ligand binding domain of PPARγ is utilized, in combination with the DNA binding domain of GAL4 protein, for the identification of PPARγ ligands or ligand-precursors. This allows one to avoid possible background signal caused by the potential presence of endogenous PPARγ in the host cells used for the assay.

The DNA binding domain of the yeast GAL4 protein comprises at least the first 74 amino acids thereof (see, for example, Keegan et al., Science 231:699-704 (1986)). Preferably, the first 90 or more amino acids of the GAL4 protein will be used, with the first 147 amino acid residues of yeast GAL4 being presently most preferred.

The GAL4 fragment employed in the practice of the present invention can be incorporated into any of a number of sites within the PPARγ receptor protein. For example, the GAL4 DNA binding domain can be introduced at the amino terminus of the PPARγ receptor protein, or the GAL4 DNA binding domain can be substituted for the native DNA binding domain of the PPARγ receptor, or the GAL4 DNA binding domain can be introduced at the carboxy terminus of the PPARγ receptor protein, or at other positions as can readily be determined by those of skill in the art. Thus, for example, a modified receptor protein can be prepared which consists essentially of amino acid residues 1-147 of GAL4, plus the ligand binding domain of PPARγ (i.e., containing the ligand binding domain only of said receptor (i.e., residues 163-475 of SEQ ID NO:1),

substantially absent the DNA binding domain and amino terminal domain thereof).

Identification methods according to the present invention involve the use of a functional bioassay system, wherein the modified receptor and a reporter plasmid are cultured in suitable host cells in the presence of test compound. Evidence of transcription (e.g., expression) of reporter gene is then monitored to determine the presence of an activated receptor-ligand complex. Accordingly, the functional bioassay system utilizes two plasmids: an "expression" plasmid and a "reporter" plasmid. The expression plasmid can be any plasmid which contains and is capable of expressing DNA encoding the desired form of PPARγ receptor protein (i.e., intact receptor or GAL4 chimeric receptor as described hereinabove), in a suitable host cell. The reporter plasmid can be any plasmid which contains an operative PPRE or GAL4 response element, as appropriate, functionally linked to an operative reporter gene.

Exemplary PPREs have been described in detail hereinabove. Exemplary GAL4 response elements are those containing the palindromic 17-mer:

5'-CGGAGGACTGTCCTCCG-3' (SEQ ID NO:6),

such as, for example, 17MX, as described by Webster et al., in Cell 52:169-178 (1988), as well as derivatives thereof. Additional examples of suitable response elements include those described by Hollenberg and Evans in Cell 55:899-906 (1988); or Webster et al. in Cell 54:199-207 (1988).

Exemplary reporter genes include chloramphenicol transferase (CAT), luciferase (LUC), beta-galactosidase (β-gal), and the like. Exemplary

promoters include the simian virus (SV) promoter or modified form thereof (e.g., ΔSV), the thymidine kinase (TK) promoter, the mammary tumor virus (MTV) promoter or modified form thereof (e.g., ΔMTV), and the like [see, for example, Mangelsdorf et al., in Nature 345:224-229 (1990), Mangelsdorf et al., in Cell 66:555-561 (1991), and Berger et al., in J. Steroid Biochem. Molec. Biol. 41:733-738 (1992)]. The plasmids pGMCAT, pGHCAT, pTK-GAL_p3-LUC, ΔMTV-GAL_p3-LUC, ΔMTV-GAL_p3-CAT, and the like, are examples of reporter plasmids which contain an operative hormone responsive promoter/enhancer element functionally linked to an operative reporter gene, and can therefore be used in the above-described functional bioassay (see Example 2 for details on the preparation of these plasmids). In pGMCAT, the operative hormone responsive promoter/enhancer element is the MTV LTR; in pGHCAT it is the functional portion of the growth hormone promoter. In both pGMCAT and GHCAT the operative reporter gene is the bacterial gene for chloramphenicol acetyltransferase (CAT).

As used herein in the phrase "operative response element functionally linked to an operative reporter gene", the word "operative" means that the respective DNA sequences (represented by the terms "PPRE," "GAL4 response element" and "reporter gene") are operational, i.e., work for their intended purposes; the word "functionally" means that after the two segments are linked, upon appropriate activation by a ligand-receptor complex, the reporter gene will be expressed as the result of the fact that the "PPRE" or "GAL4 response element" was "turned on" or otherwise activated.

In practicing the above-described functional bioassay, the expression plasmid and the reporter plasmid are co-transfected into suitable host cells. The transfected host cells are then cultured in the presence

and absence of a test compound to determine if the test compound is able to produce activation of the promoter operatively linked to the PPRE or GAL4 response element of the reporter plasmid. Thereafter, the transfected and cultured host cells are monitored for induction (i.e., the presence) of the product of the reporter gene sequence.

Any cell line can be used as a suitable "host" for the functional bioassay contemplated for use in the practice of the present invention. Thus, in contrast to the requirements of prior art assay systems, when GAL4 chimerics are employed, there is no need to use receptor-negative cells in carrying out the invention process. Since the modified receptor employed in the practice of the present invention is the only species in the test cell which is capable of initiating transcription from a GAL4 response element, the expression of native receptor by the test cell does not contribute to background levels. Thus, the invention bioassay can be made to be very selective.

Cells contemplated for use in the practice of the present invention include transformed cells, non-transformed cells, neoplastic cells, primary cultures of different cell types, and the like. Exemplary cells which can be employed in the practice of the present invention include Schneider cells, CV-1 cells, HuTu80 cells, F9 cells, NTERA2 cells, NB4 cells, HL-60 cells, 293 cells, Hela cells, yeast cells, and the like. Preferred host cells for use in the functional bioassay system are COS cells and CV-1 cells. COS-1 (referred to as COS) cells are monkey kidney cells that express SV40 T antigen (Tag); while CV-1 cells do not express SV40 Tag. The presence of Tag in the COS-1 derivative lines allows the introduced expression plasmid to replicate and provides a relative increase in the amount of receptor

produced during the assay period. CV-1 cells are presently preferred because they are particularly convenient for gene transfer studies and provide a sensitive and well-described host cell system.

The above-described cells (or fractions thereof) are maintained under physiological conditions when contacted with physiologically active compound. "Physiological conditions" are readily understood by those of skill in the art to comprise an isotonic, aqueous nutrient medium at a temperature of about 37°C.

In accordance with another embodiment of the present invention, there is provided a method of screening for antagonists of PPARγ receptor proteins, said method comprising

culturing test cells containing

(i) increasing concentrations of at least one compound whose ability to inhibit the transcription activation activity of PPARγ agonists is sought to be determined, and

(ii) optionally, at least one PPARγ agonist,

wherein said test cells contain

(i) exogenous DNA which expresses intact PPARγ or a modified form of PPARγ, wherein the modified form of PPARγ contains the DNA binding domain of GAL4, and

(ii) a PPRE or GAL4 response element, respectively, operatively linked to a reporter gene; and thereafter

assaying for evidence of transcription of said reporter gene in said cells as a function of the concentration of said compound in said culture medium, thereby indicating the ability of said compound to inhibit activation of transcription by PPARγ agonists.

Media employed for such culturing may include agonist for the receptor being tested, or the receptor may be constitutive (i.e., not require the presence of agonist for activation), or a fixed concentration of agonist can be added to the media employed for such testing.

The above-described assays of the present invention have low background and a broad dynamic range.

In accordance with yet another embodiment of the present invention, there is provided a method for preventing obesity, said method comprising administering to a subject in need thereof an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) antagonist effective to block cell differentiation to produce lipid-accumulating cells.

As employed here, "obesity" refers generally to individuals who are at least about 20-30% over the average weight for his/her age, sex and height. Technically, "obese" is defined, for males, as individuals whose body mass index is greater than 27.8 kg/m^2, and for females, as individuals whose body mass index is greater than 27.3 kg/m^2.

Those of skill in the art recognize that there are numerous cell types which are capable of differentiation to produce "lipid-accumulating cells,"

such as, for example, mesenchymal cells (e.g., fibroblasts).

As employed herein, the phrase "amount... effective to block cell differentiation" refers to levels of compound sufficient to provide circulating concentrations high enough to effect activation of PPARγ. Such a concentration typically falls in the range of about 10 nM up to 2 μM; with concentrations in the range of about 100 nM up to 200 nM being preferred.

In accordance with a particular embodiment of the present invention, compositions comprising at least one prostaglandin or prostaglandin-like compound (as described above), and a pharmaceutically acceptable carrier are contemplated. Exemplary pharmaceutically acceptable carriers include carriers suitable for oral, intravenous, subcutaneous, intramuscular, intracutaneous, and the like administration. Administration in the form of creams, lotions, tablets, dispersible powders, granules, syrups, elixirs, sterile aqueous or non-aqueous solutions, suspensions or emulsions, and the like, is contemplated.

For the preparation of oral liquids, suitable carriers include emulsions, solutions, suspensions, syrups, and the like, optionally containing additives such as wetting agents, emulsifying and suspending agents, sweetening, flavoring and perfuming agents, and the like.

For the preparation of fluids for parenteral administration, suitable carriers include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable

organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized, for example, by filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile water, or some other sterile injectable medium immediately before use.

In accordance with still another embodiment of the present invention, there is provided a method for treating diabetes, said method comprising administering to a subject in need thereof an amount of a peroxisome proliferator activated receptor-gamma (PPAR-γ) agonist effective to lower the blood glucose level of said subject.

As employed herein, the phrase "amount... effective to lower blood glucose levels" refers to levels of compound sufficient to provide circulating concentrations high enough to accomplish the desired effect. Such a concentration typically falls in the range of about 10 nM up to 2 μM; with concentrations in the range of about 100 nM up to 200 nM being preferred.

The invention will now be described in greater detail by reference to the following non-limiting examples.

Example 1

Preparation of GAL4-receptor fusion proteins

A basic vector useful for the generation of GAL4-receptor fusion proteins is called pCMX-GAL4 (see SEQ ID NO:2). This vector encodes GAL4 DNA binding

domain, followed by a polylinker sequence useful in the cloning. The parental expression vector pCMX has been described by Umesono et al., in Cell 65:1255-1266 (1991), and the GAL4 portion of pCMX-GAL4 is derived from plasmid pSG424, described by Sadowski and Ptashne, in Nucleic Acids Res. 17:7539 (1989).

In general, GAL4-receptor ligand binding domain fusions are prepared by taking advantage of mutant receptor cDNA clones, such as GR-RAR chimera [see, for example, Giguere et al., in Nature 330:624-629 (1987)]. These mutant receptor cDNAs encode common *Xho*I sites at the end of the DNA binding domain, as described by Giguere et al., supra. To do so, a new pCMX-GAL4 vector was prepared which encodes a compatible *Sal*I site in the polylinker sequence (there is an *Xho*I site in the GAL4 sequence):

*Sal*I site: G'TCGAC

*Xho*I site: C'TCGAG

This allows efficient transfer of the receptor ligand binding domain to GAL4 DNA binding domain. Through this method, a number of chimeric species have been generated, including GAL4-PPARγ, containing residues 163-475 of PPARγ (see SEQ ID NO:1).

If mutants of the type referred to above are not available for the construction of GAL4-containing chimerics, one may simply look for any convenient restriction enzyme site within or downstream of the DNA binding domain of the receptor of interest (i.e., within about the first 30 amino acid residues downstream of the conserved Gly-Met residues of the DNA binding domain, i.e., within 30 residues of the last two residues shown in SEQ ID NO:1), and utilize the carboxy terminal sequences therefrom.

Example 2
Preparation of reporter constructs

Various reporter constructs are used in the examples which follow. They are prepared as follows:

TK-LUC: The MTV-LTR promoter sequence was removed from the MTV-LUC plasmid described by Hollenberg and Evans in Cell 55:899-906 (1988) by *Hin*dIII and *Xho*I digest, and cloned with the *Hin*dIII-*Xho*I fragment of the Herpes simplex virus thymidine kinase gene promoter (-105 to +51 with respect to the transcription start site, m, isolated from plasmid pBLCAT2, described by Luckow & Schutz in Nucleic Acids Res. 15:5490 (1987)) to generate parental construct TK-LUC.

pTK-PPRE3-LUC: Three copies of double-stranded peroxisome proliferator response element (PPRE) oligonucleotides (see SEQ ID NO:3) were cloned upstream of the TK promoter of TK-LUC at the *Sal*I site.

pTK-MH100x4-LUC: Four copies of double-stranded MH100 oligonucleotides, encoding a GAL4 binding site, were cloned upstream of the TK promoter of TK-LUC at the *Hin*dIII site.

CMX-βGAL: The coding sequence for the *E. coli* β-galactosidase gene was isolated from plasmid pCH110 [see Hall et al., J. Mol. Appl. Genet. 2:101-109 (1983)] by *Hin*dIII and *Bam*HI digest, and cloned into pCMX eucaryotic expression vector [see Umesono et al., supra].

Example 3
Screening assay for receptor selective agonists

CV-1 cells are co-transfected with CMX-GAL-PPARγ and pTK-MH100x4-LUC at a ratio of about 100 ng of

receptor-encoding DNA per 10^5 cells. The usual amounts of DNA per 10^5 cells are 100 ng of CMX-GAL-PPARγ, 300 ng of pTK-MH100x4-LUC, and 500 ng of CMX-βGAL. Typically, transfections are performed in triplicate. The plates are then incubated for 2-3 hours at 37°C.

The cells are washed with fresh medium. Fresh medium containing one concentration of a serial dilution of agonist is added to each well. A typical agonist dilution series extends from 10^{-5}M through 10^{-11}M. A solvent control is performed for each agonist. The cells are incubated at 37°C for 1-2 days.

The cells are rinsed twice with buffered saline solution. Subsequently, cells are lysed, *in situ*, by adding 200 μl of lysis buffer. After 30 minutes incubation at room temperature, 40 μl aliquots of cell lysate are transferred to 96-well plates for luciferase reporter gene assays and β-galactosidase transfection controls [see Heyman et al., Cell 68:397-406 (1992)].

The data are expressed as relative light units (RLUs) per O.D. unit of β-galactosidase per minute. The triplicates are averaged for each concentration and plotted (see Figure 1) as fold induction induced by a standard dose (10μM) of agonist.

Example 4

Dose response of GAL4-PPARγ constructs to various prostaglandins

Effector plasmid, reporter plasmid, and β-galactosidase control plasmid are co-transfected into CV-1 cells at a ratio of about 1:3:5, using a liposome-mediated method, employing N-{2-(2,3)-dioleoyloxy)propyl-N,N,N-trimethyl ammonium methyl sulfate} (i.e., DOTAP, Boehringer Mannheim) according to the manufacturer's

instructions in Dulbecco's modified Eagle's medium (DMEM) with 10% delipidated hormone-depleted fetal calf serum. After about 2-3 hours, the cells are washed with DMEM and an appropriate prostaglandin is added to the media to the final molar concentration indicated in Figure 2. After 24-48 hours of incubation, the cells are rinsed with phosphate buffered saline (pH 7.2) and lysed. Aliquots are assayed for luciferase and β-galactosidase activity. Luciferase activity is normalized to optical density units of β-galactosidase per minute of incubation.

The data are expressed in Figure 2 as fold induction over the same construct incubated in solvent alone. Review of Figure 2 reveals that PGD2 and PGJ2 families of compounds are functional modulators of PPARγ.

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and claimed.